Supplementary Information

An enzymatic route to a benzocarbazole framework using bacterial CotA laccase

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1. General experimental information:

2,4-diaminodiphenylamine (2,4-DADPA), was purchased from Aldrich as a violet solid and used without further purification. Structural assignments were based on 1D-NMR (^{1}H,^{13}C) e 2D-NMR (COSY, HSQC and HMBC) spectra that were obtained in Acetone-d_6 on a Bruker Advance 400MHz spectrometer. Chemical shifts are reported in ppm relative to the solvent peak and coupling constants (*J*) are reported in Hertz. FTIR spectra were obtained in KBr pellets on a Bruker Vertex 70 FT-IR spectrometer. ESI-MS spectra were acquired in full scan mode (m/z) on a 500-MS LC ion trap mass spectrometer (Varian, Inc., Palo Alto, CA), operated in the positive electrospray ionization (ESI) mode. The optimized operating parameters were: ion spray voltage: ±5 kV; capillary voltage: 20 V and RF loading: 90%. High resolution mass measurements was performed on a MicrOTOF equipped with an ESI source (Bruker, Daltonics) at Unidade de Spectrometria, Universidade de Santiago de Compostela.

The redox potential of 2,4-DADPA was obtained by cyclic voltammetry using an EG&G Princeton Applied Research Model 273A potentiostat/galvanostat monitored with a personal computer loaded with Electrochemistry PowerSuite v2.51 software from Princeton Applied Research. Cyclic voltammograms were obtained with 1mM of substrate in 1:9 MeOH:100 mM buffer (phosphate for pH ≥ 6 and Britton-Robinson for pH 4) solutions using a three-electrode configuration cell with an home-made platinum- disk working electrode (1.0mm diameter), a platinum wire counter electrode and a saturated calomel reference electrode (SCE) (purchased from Radiometer analytical, SAS, France). The potential was scanned from -0.7 to 1.2 V at a scan rate of 100 mV/s. All measurements were done at room temperature and the solutions were deaerated with N_2 before use. The measured potentials were corrected by +0.197 V to the normal hydrogen electrode (NHE).

The molar extinction coefficient for compound (**1)** was determined in the concentration range: 2.5×10^{-4} - 5.0×10^{-4}M in a BioTek Synergy 2 spectrophotometer. The kinetic parameters for 2,4-DADPA (0.05–3 mM) were measured in Britton-Robinson buffer, pH 6 (100 mM phosphoric acid, 100 mM boric acid and 100 mM acetic acid mixed with NaOH to the desired pH) at 37 °C. Kinetic constants (K_m and k_{cat}) were fitted directly using the Michaelis–Menten equation (OriginLab software, Northampton, MS, USA). All enzymatic assays were performed at least in triplicate.

2. X-ray crystal structure data

Crystals suitable for X-ray analysis were obtained by slow evaporation of saturated solutions in acetone. The crystal was mounted on a loop with Fomblin protective oil and the X-ray data was collected on Bruker AXS-KAPPA APEX II diffractometer with graphite monochromated radiation (Mo Kα, λ = 0.71069 Å) at 150 K. The X-ray generator was operated at 50 kV and 30 mA, and the X-ray data collection was monitored by the APEX2[1] program. All data were corrected for Lorentzian, polarization, and absorption effects using SAINT[1] and SADABS[2] programs.

SIR97[3] were used for structure solution, and SHELXL-97[4] was used for full matrix least-squares refinement on F^2.

These two programs are included in the package of programs WINGX-Version 1.80.05.[5] A full-matrix least-squares refinement was used for the non-hydrogen atoms with anisotropic thermal parameters.

All the hydrogens were inserted in idealized positions and allowed to refine riding in the parent carbon atom, except the N5 nitrogen hydrogen atom that was found in the electron density map.

The graphics were made using MERCURY 3.0[6]. PLATON[7] was used for hydrogen bond and intermolecular interactions. Although the data collection was done at low temperature the quality of the data is poor due to the crystal quality.

Table S1 summarizes crystallographic details for compound **1** and Tables S2 and S3 the bond lengths, bond angles and torsion angles respectively.

CCDC 1040890 contain the Cif file for this compound. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk

The crystal structure determination displays one molecule of **2** and one molecule of acetone in the asymmetric unit.

The crystal packing diagram of this compound is characterized by one dimensional infinite chain $C_1^1(6)$, along the b axis, that is sustained by the short N3-H3N…N4 hydrogen bond (2.01Å). The solvent molecule interacts with the N1 atom through C100–H10C···N1 [2.62Å] non-classical intermolecular bond. Consecutive herring bone chains are linked one to another by C25–H25···O110 (2.61Å) intermolecular interactions, that are reinforced by C-H…π interaction (3.33 Å) between a methyl group of the solvent and a benzenoid ring, giving rise to a 3D herring bone pattern as can be seen in figure S1.

Compound **2**:

Table S1. Crystal Data and structure refinement details for compound **2:**

Empirical Formula	$C_{33}H_{33}N_5O$
Formula weight	515.40
Crystal system	Monoclinic
Space group	P2(1)/n
a ,b, c [Å]	11.9187(17), 9.8408(15), 24.353(4)
α, β, γ [º]	90, 102.942(6), 90
V [Å^3]	2783.8(7)
Z	4
D_{calc} (Mg/m^3)	1.230
Temperature (K)	150(2)
MoKα (Å)	0.71073
μ (Mo Kα) (mm^{-1})	0.076
Number of reflections	21887

Number of unique data	5915
R_{int}	0.1210
Final R_1[a]	0.0681
ωR_2 [b] ($I \geq 2\delta$)	0.1345
Goodness-of-fit (GOF) on F^2	0.838
Extinction coefficient	0.0097(10)
Largest diff. peak and hole [e. $Å^{-3}$]	0.268 and -0.244

Table S2. Bond lengths [Å] and angles [deg] for compound **2**

Bond Lengths			
N(3)-C(6)	1.385(4)	N(3)-C(8)	1.384(4)
N(5)-C(12)	1.388(3)	N(5)-C(51)	1.428(3)
N(5)-C(15)	1.499(4)	N(4)-C(13)	1.301(4)
N(4)-C(15)	1.457(3)	N(1)-C(2)	1.294(4)
N(1)-C(1)	1.466(4)	N(2)-C(3)	1.355(4)
N(2)-C(21)	1.430(4)	N(2)-C(1)	1.485(4)
C(8)-C(14)	1.351(4)	C(8)-C(9)	1.454(4)
C(12)-C(11)	1.360(4)	C(12)-C(13)	1.463(4)
C(13)-C(14)	1.436(4)	C(6)-C(7)	1.350(4)
C(6)-C(5)	1.461(4)	C(4)-C(3)	1.365(4)
C(4)-C(5)	1.425(4)	C(5)-C(9)	1.388(4)
C(2)-C(7)	1.430(4)	C(2)-C(3)	1.470(4)
C(9)-C(11)	1.432(4)	C(51)-C(56)	1.383(4)
C(51)-C(52)	1.387(4)	C(15)-C(151)	1.517(4)
C(15)-C(152)	1.534(4)	C(56)-C(55)	1.391(4)
C(52)-C(53)	1.384(4)	C(21)-C(26)	1.370(4)
C(21)-C(22)	1.382(4)	C(53)-C(54)	1.380(4)
C(1)-C(112)	1.504(4)	C(1)-C(111)	1.523(5)
C(55)-C(54)	1.381(4)	C(24)-C(23)	1.362(5)
C(24)-C(25)	1.379(4)	C(26)-C(25)	1.398(4)
C(23)-C(22)	1.384(4)	C(101)-C(100)	1.479(5)

C(101)-C(02)	1.485(5)	O(100)-C(101)	1.204(4)
Bond Angles			
C(6)-N(3)-C(8)	109.0(3)	C(12)-N(5)-C(51)	123.2(2)
C(12)-N(5)-C(15)	108.6(2)	C(51)-N(5)-C(15)	122.6(2)
C(13)-N(4)-C(15)	108.9(2)	C(2)-N(1)-C(1)	108.3(2)
C(3)-N(2)-C(21)	124.2(2)	C(3)-N(2)-C(1)	110.2(2)
C(21)-N(2)-C(1)	124.5(2)	C(14)-C(8)-N(3)	127.6(3)
C(14)-C(8)-C(9)	123.8(3)	N(3)-C(8)-C(9)	108.6(3)
C(11)-C(12)-N(5)	132.7(3)	C(11)-C(12)-C(13)	122.2(3)
N(5)-C(12)-C(13)	105.0(2)	N(4)-C(13)-C(14)	126.4(3)
N(4)-C(13)-C(12)	113.0(2)	C(14)-C(13)-C(12)	120.5(3)
C(7)-C(6)-N(3)	127.7(3)	C(7)-C(6)-C(5)	124.4(3)
N(3)-C(6)-C(5)	107.9(3)	C(3)-C(4)-C(5)	117.0(3)
C(9)-C(5)-C(4)	133.1(3)	C(9)-C(5)-C(6)	107.5(2)
C(4)-C(5)-C(6)	119.3(3)	C(8)-C(14)-C(13)	116.4(3)
N(1)-C(2)-C(7)	127.3(3)	N(1)-C(2)-C(3)	113.1(3)
C(7)-C(2)-C(3)	119.7(3)	C(5)-C(9)-C(11)	133.2(3)
C(5)-C(9)-C(8)	107.0(2)	C(11)-C(9)-C(8)	119.8(3)
C(12)-C(11)-C(9)	117.3(3)	C(56)-C(51)-C(52)	118.9(3)
C(56)-C(51)-N(5)	120.8(3)	C(52)-C(51)-N(5)	120.3(3)
N(2)-C(3)-C(4)	132.3(3)	N(2)-C(3)-C(2)	104.6(2)
C(4)-C(3)-C(2)	123.1(3)	C(6)-C(7)-C(2)	116.5(3)
N(4)-C(15)-N(5)	104.4(2)	N(4)-C(15)-C(151)	109.6(3)
N(5)-C(15)-C(151)	112.3(2)	N(4)-C(15)-C(152)	106.7(2)
N(5)-C(15)-C(152)	112.0(3)	C(151)-C(15)-C(152)	111.4(3)
C(51)-C(56)-C(55)	120.7(3)	C(53)-C(52)-C(51)	120.3(3)
C(26)-C(21)-C(22)	119.5(3)	C(26)-C(21)-N(2)	121.3(3)
C(22)-C(21)-N(2)	119.2(3)	C(52)-C(53)-C(54)	120.7(3)
N(1)-C(1)-N(2)	103.7(2)	N(1)-C(1)-C(112)	110.1(2)
N(2)-C(1)-C(112)	110.9(3)	N(1)-C(1)-C(111)	108.6(3)
N(2)-C(1)-C(111)	111.2(2)	C(112)-C(1)-C(111)	111.9(3)
C(54)-C(55)-C(56)	120.1(3)	C(23)-C(24)-C(25)	120.1(3)
C(21)-C(26)-C(25)	120.7(3)	C(24)-C(25)-C(26)	119.1(3)
C(53)-C(54)-C(55)	119.3(3)	C(24)-C(23)-C(22)	120.8(3)

C(23)-C(22)-C(21)	119.8(3)	O(100)-C(101)-C(100)	121.5(4)
O(100)-C(101)-C(02)	121.5(4)	C(100)-C(101)-C(02)	117.0(3)

Table S3. Torsion angles [deg] for compound **2**

C(6)-N(3)-C(8)-C(14)	-178.4(3)	C(6)-N(3)-C(8)-C(9)	2.3(3)
C(51)-N(5)-C(12)-C(11)	-27.0(5)	C(15)-N(5)-C(12)-C(11)	178.8(3)
C(51)-N(5)-C(12)-C(13)	154.9(3)	C(15)-N(5)-C(12)-C(13)	0.8(3)
C(15)-N(4)-C(13)-C(14)	179.8(3)	C(15)-N(4)-C(13)-C(12)	-0.3(3)
C(11)-C(12)-C(13)-N(4)	-178.6(3)	N(5)-C(12)-C(13)-N(4)	-0.3(3)
C(11)-C(12)-C(13)-C(14)	1.3(4)	N(5)-C(12)-C(13)-C(14)	179.6(2)
C(8)-N(3)-C(6)-C(7)	179.7(3)	C(8)-N(3)-C(6)-C(5)	-1.4(3)
C(3)-C(4)-C(5)-C(9)	-178.7(3)	C(3)-C(4)-C(5)-C(6)	0.2(4)
C(7)-C(6)-C(5)-C(9)	178.8(3)	N(3)-C(6)-C(5)-C(9)	-0.1(3)
C(7)-C(6)-C(5)-C(4)	-0.3(4)	N(3)-C(6)-C(5)-C(4)	-179.2(3)
N(3)-C(8)-C(14)-C(13)	-179.7(3)	C(9)-C(8)-C(14)-C(13)	-0.5(4)
N(4)-C(13)-C(14)-C(8)	179.7(3)	C(12)-C(13)-C(14)-C(8)	-0.2(4)
C(1)-N(1)-C(2)-C(7)	-178.3(3)	C(1)-N(1)-C(2)-C(3)	2.1(3)
C(4)-C(5)-C(9)-C(11)	-1.9(6)	C(6)-C(5)-C(9)-C(11)	179.1(3)
C(4)-C(5)-C(9)-C(8)	-179.6(3)	C(6)-C(5)-C(9)-C(8)	1.4(3)
C(14)-C(8)-C(9)-C(5)	178.3(3)	N(3)-C(8)-C(9)-C(5)	-2.3(3)
C(14)-C(8)-C(9)-C(11)	0.3(4)	N(3)-C(8)-C(9)-C(11)	179.6(2)
N(5)-C(12)-C(11)-C(9)	-179.3(3)	C(13)-C(12)-C(11)-C(9)	-1.5(4)
C(5)-C(9)-C(11)-C(12)	-176.7(3)	C(8)-C(9)-C(11)-C(12)	0.7(4)
C(12)-N(5)-C(51)-C(56)	-26.7(4)	C(15)-N(5)-C(51)-C(56)	123.8(3)
C(12)-N(5)-C(51)-C(52)	153.9(3)	C(15)-N(5)-C(51)-C(52)	-55.5(4)
C(21)-N(2)-C(3)-C(4)	10.3(5)	C(1)-N(2)-C(3)-C(4)	178.9(3)
C(21)-N(2)-C(3)-C(2)	-171.3(3)	C(1)-N(2)-C(3)-C(2)	-2.8(3)
C(5)-C(4)-C(3)-N(2)	178.4(3)	C(5)-C(4)-C(3)-C(2)	0.3(4)
N(1)-C(2)-C(3)-N(2)	0.4(3)	C(7)-C(2)-C(3)-N(2)	-179.2(3)
N(1)-C(2)-C(3)-C(4)	179.0(3)	C(7)-C(2)-C(3)-C(4)	-0.7(4)
N(3)-C(6)-C(7)-C(2)	178.6(3)	C(5)-C(6)-C(7)-C(2)	-0.1(4)
N(1)-C(2)-C(7)-C(6)	-179.1(3)	C(3)-C(2)-C(7)-C(6)	0.6(4)
C(13)-N(4)-C(15)-N(5)	0.8(3)	C(13)-N(4)-C(15)-C(151)	-119.7(3)

C(13)-N(4)-C(15)-C(152)	119.5(3)	C(12)-N(5)-C(15)-N(4)	-1.0(3)
C(51)-N(5)-C(15)-N(4)	-155.3(2)	C(12)-N(5)-C(15)-C(151)	117.7(3)
C(51)-N(5)-C(15)-C(151)	-36.6(4)	C(12)-N(5)-C(15)-C(152)	-116.0(3)
C(51)-N(5)-C(15)-C(152)	89.7(3)	C(52)-C(51)-C(56)-C(55)	-0.5(5)
N(5)-C(51)-C(56)-C(55)	-179.9(3)	C(56)-C(51)-C(52)-C(53)	-1.0(5)
N(5)-C(51)-C(52)-C(53)	178.4(3)	C(3)-N(2)-C(21)-C(26)	-105.6(4)
C(1)-N(2)-C(21)-C(26)	87.5(4)	C(3)-N(2)-C(21)-C(22)	75.5(4)
C(1)-N(2)-C(21)-C(22)	-91.4(4)	C(51)-C(52)-C(53)-C(54)	2.3(5)
C(2)-N(1)-C(1)-N(2)	-3.6(3)	C(2)-N(1)-C(1)-C(112)	-122.3(3)
C(2)-N(1)-C(1)-C(111)	114.8(3)	C(3)-N(2)-C(1)-N(1)	3.9(3)
C(21)-N(2)-C(1)-N(1)	172.4(3)	C(3)-N(2)-C(1)-C(112)	122.1(3)
C(21)-N(2)-C(1)-C(112)	-69.4(4)	C(3)-N(2)-C(1)-C(111)	-112.6(3)
C(21)-N(2)-C(1)-C(111)	55.9(4)	C(51)-C(56)-C(55)-C(54)	0.8(5)
C(22)-C(21)-C(26)-C(25)	-1.3(5)	N(2)-C(21)-C(26)-C(25)	179.8(3)
C(23)-C(24)-C(25)-C(26)	0.5(5)	C(21)-C(26)-C(25)-C(24)	0.6(5)
C(52)-C(53)-C(54)-C(55)	-2.0(5)	C(56)-C(55)-C(54)-C(53)	0.5(5)
C(25)-C(24)-C(23)-C(22)	-1.0(5)	C(24)-C(23)-C(22)-C(21)	0.4(5)
C(26)-C(21)-C(22)-C(23)	0.8(5)	N(2)-C(21)-C(22)-C(23)	179.7(3)

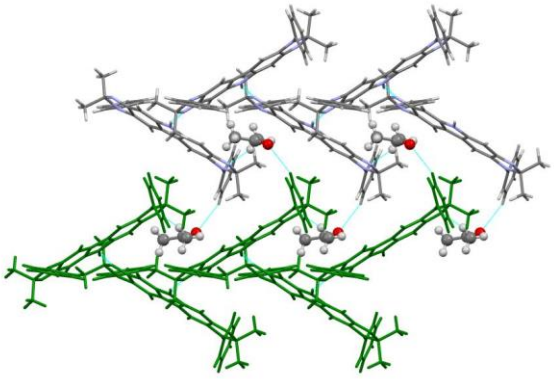

Figure S2.1– View of three-dimensional packing pattern of compound ***2***

[1]- SAINT+, release 6.22; Bruker Analytical Systems: Madison,WI, 2005.

[2] - SADABS; Bruker Analytical Systems: Madison,WI, 2005.

[3] – A. Altomare, M.C. Burla, M. Camalli, G.L. Cascarano, C. Giacovazzo, A. Guagliardi, A.G.G. Moliterni, G. Polidori and R. Spagna, *J. Appl. Crystallogr.*, 1999, **32**, 115.

[4] – G.M. Sheldrick, *Acta Crystallogr., Sect. A*, 2008, **64**, 112.

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[6] – C.F. Macrae, I.J. Bruno, J.A. Chisholm, P.R. Edgington, P. McCabe, E. Pidcock, L. Rodriguez-Monge, R. Taylor, J. van de Streek and P.A. Wood, *J. Appl. Crystallogr.*, 2008, **41**, 466.

[7] – A.L. Spek, *Acta Crystallogr. Sect. D: Biol. Crystallogr.*, 2009, **65**, 148.

3. NMR and MS spectra of compounds 1 and 2

Compound **1:**

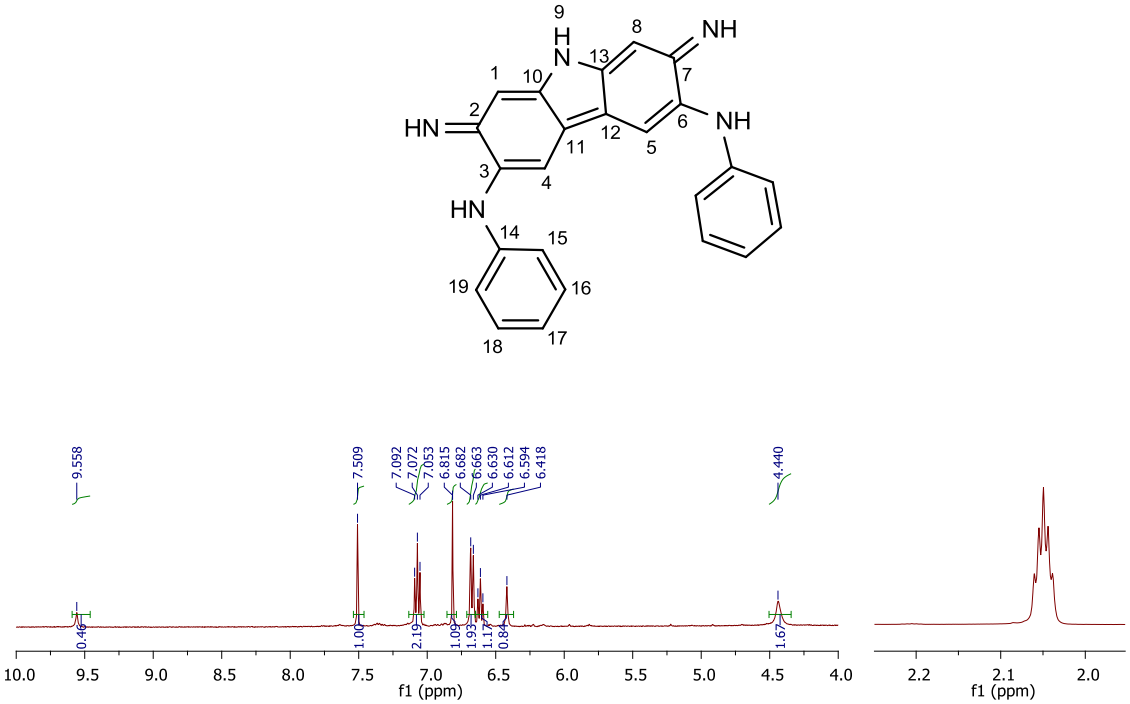


Figure S3.1 - ^{1}H-NMR spectrum of compound **1** in $(CD_3)_2CO$ (400MHz), with expansion of solvent region

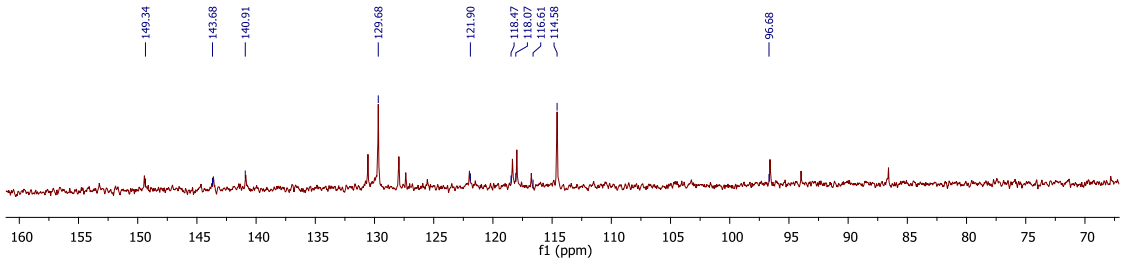


Figure S3.2 – ^{13}C-NMR spectrum of compound **1** in $(CD_3)_2CO$ (400MHz)

ESI(+)/MS spectrum of compound **1** ($C_{24}H_{19}N_5$) MW=377.44 g/mol

Positive mode *m/z* = 378 $[M+H]^+$

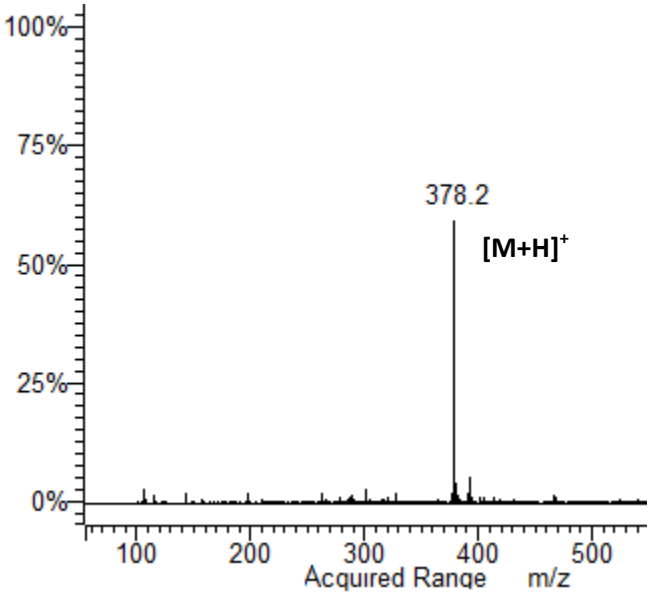


Figure S3.3- ESI(+)/MS spectrum of compound **1**

ESI(+)-MS^2 spectrum:

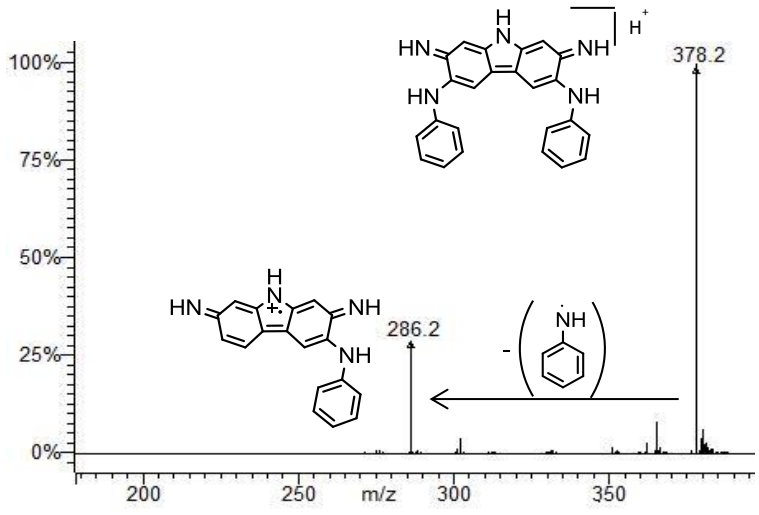


Figure S3.4- ESI(+)/MS_2 spectrum of protonated ion *m/z* 378

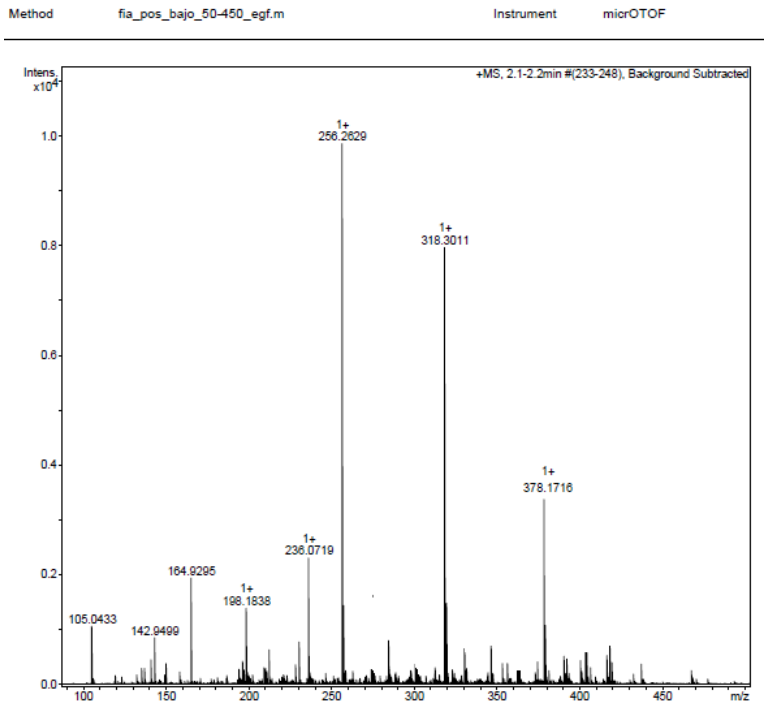
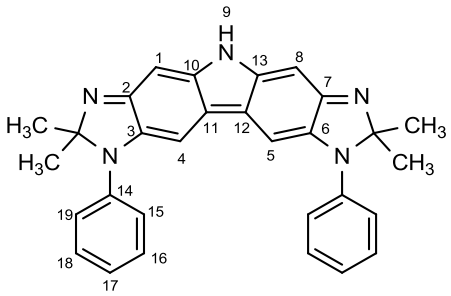


Figure S3.5 - HR-MS spectrum (ESI-FIA-TOF) of compound **1**

Compound **2:**



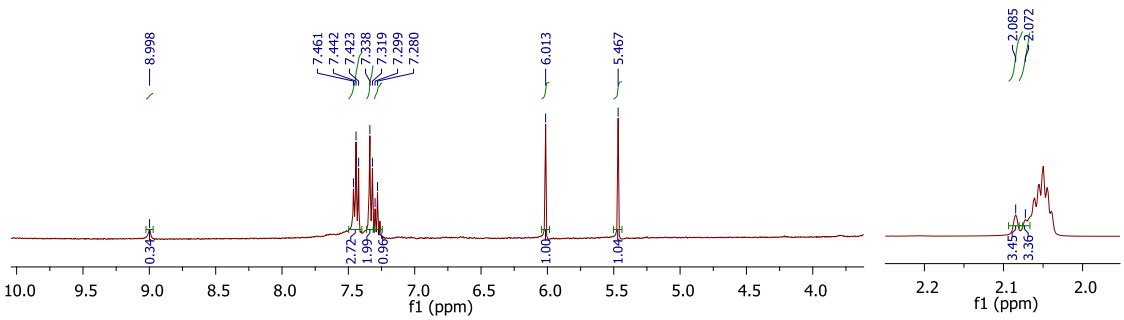


Figure S3.6 - ^{1}H-NMR spectrum of compound ***2*** in $(CD_3)_2CO$ (400MHz) with expansion of solvent region

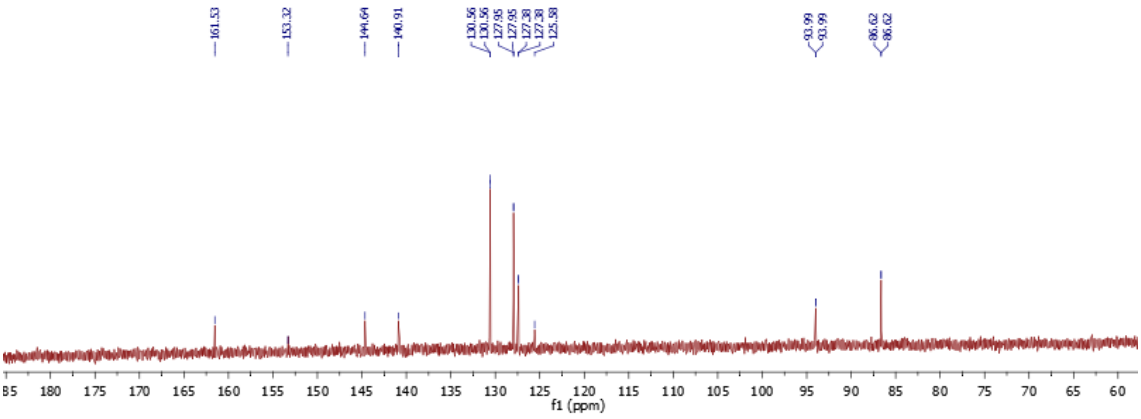


Figure S3.7 – ^{13}C-NMR spectrum of compound ***2*** in $(CD_3)_2CO$ (400MHz)

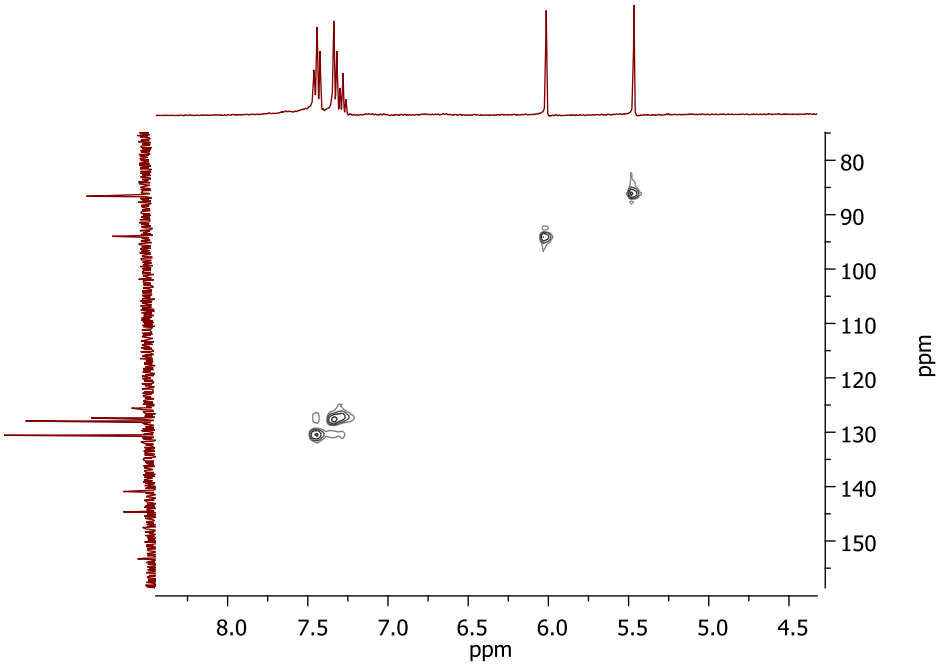


Figure S3.8 – HSQC-NMR spectrum of compound ***2*** in $(CD_3)_2CO$ (400MHz)

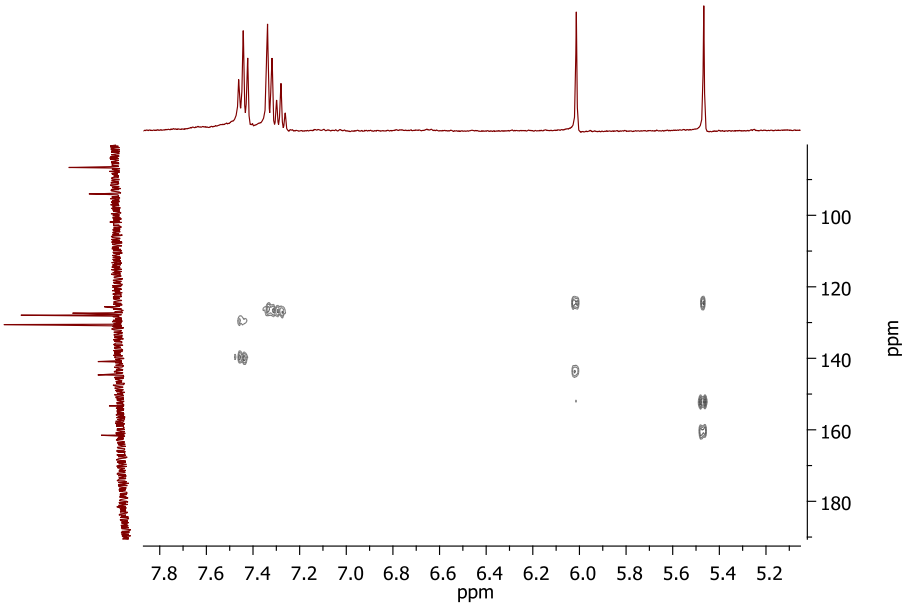


Figure S3.9 – HMBC-NMR spectrum of compound ***2*** in $(CD_3)_2CO$ (400MHz)